

Effect of different media on mycelium growth of *Sclerotium rolfsii* Sacc. *invitro* condition

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Abstract— An *invitro* experiment was conducted to study the effect of different culture media on mycelial growth of *Sclerotium rolfsii* Sacc. in Plant pathology Laboratory of Agriculture and Forestry University, Nepal. The pathogenic isolate of *S. rolfsii* was isolated from symptomatic tomato and maintained in PDA. Seven different culture media viz. Potato Dextrose Agar (PDA), Chickpea Dextrose Agar (ChDA), Bean Dextrose Agar (BDA), Carrot Dextrose Agar (CDA), Papaya Dextrose Agar (PpDA), Czapek Dox Agar (CzDA) and Sabouraud's Dextrose Agar (SDA) were prepared and 5-mm diameter mycelial plugs from the margins of 3-days old *S. rolfsii* culture was transfer in the middle of each media plate. The radial mycelial growth was measured at 2 days interval for 14 days. Chickpea Dextrose Agar (ChDA) had significantly higher mycelia growth, which was superior than Potato dextrose agar (PDA). Papaya fruit, carrot root, chickpea grain and bean grain can be the common cheap source of carbon in industrial development of culture media.

Keywords— *Media, S. rolfsii, Mycelium, Carbon, isolate.*

I. INTRODUCTION

Sclerotium rolfsii Sacc. is a necrotrophic soilborne plant pathogen forming sclerotia as a survival structure distributed worldwide. It causes various symptoms like seedling damping-off, root rot, collar rot, and stem rot in more than 500 plant species commonly legumes, crucifers, and cucurbits (Barnett and Hunter, 1972). Because of prolific growth of *S. rolfsii* and ability to produce persistent sclerotia, it is contributing in high degree of economic losses (Mahen et al., 1995). Under conducive conditions it can causes 55-95% mortality of the crop at seedling stage (Gurha and Dubey, 1982). Infection on plants by the pathogen is governed and triggered by the initial recognition phenomenon. Establishment of the pathogen in host depends upon some kind of similarity between two interacting partners (De Vay & Adler, 1976).

The capacity of fungi to utilize the available nutrients, of which carbohydrates are the major ones determine the ability of fungi to grow in different media. So it was observed that the growth rate varies with different carbon sources. Fungal media containing high carbohydrate source, nitrogen source is required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37°C. Fungal culture media are natural and synthetic. Natural media are composed of natural substrates like

potato dextrose agar, V-8 juice agar etc whereas synthetic media contain defined amounts of carbohydrates, nitrogen, and vitamin sources (eg: Czapek-Dox medium).

Potato dextrose agar (PDA) is most commonly used natural media for the culture of fungi. Most lab used media are not cost effective at a large-scale, so industrialists have started to use several common cheap sources of carbon. So, the objective of this research was to identify alternative natural fungal media using substrate with cheap source of carbon.

II. MATERIALS AND METHODS

Media preparation

Five natural media: Potato Dextrose Agar(PDA), Chickpea Dextrose Agar(ChDA), Carrot Dextrose Agar(CDA), Bean Dextrose Agar(BDA), Papaya Dextrose Agar(PpDA) and two synthetic media: Saubards Dextrose Agar(SDA),Czepadox Dextrose Agar(CzDA) were prepared. 200 g of potato pieces, chickpea grains, carrot pieces, bean grains, papaya fruit pieces were weighed individually for their respective media viz. PDA, ChDA, CDA, BDA, PpDA respectively and boiled in 500 ml of water for 10 minutes and filtered with muslin cloths. We added 20 g of dextrose and 20g of agar in each media and added distilled water to make the final volume of 1000 ml.

The synthetic media were prepared as manufacutural instruction. SDA was prepared by mixing 40 g of dextrose, 10 g of Peptone and 15 g of Agar in 1000 ml of distilled water and CzDA by 30 g of Sucrose, 2 g of sodium nitrate, 1 g of Dipotassium phosphate, 0.5 g of Magnesium

sulphate, 0.5 g of Potassium chloride, 0.01 g of Ferrous nitrate, 15 g of agar in 1000 ml of distilled water. The media were autoclaved for 121°C for 20 minutes at 15 psi pressure.

Table 1: Composition of different natural culture media

Ingredients	PDA	ChDA	PpDA	BDA	CDA
Pieces/grain	200 g				
Dextrose	20 g				
Agar	20 g				
Distilled water	1000 ml				

Isolation of *S. rolfsii*

The pathogen, *S. rolfsii* was isolated from the stems of infected tomato plants grown in Chitwan, Nepal by tissue segment method (Rangaswami and Mahadevan, 1999) on PDA. Small pieces of tissue of about 1 cm from infected collar region with some healthy tissue were cut with sterile blade. The pieces were surface sterilized with 1% sodium hypochlorite solution for 1 minute followed by three subsequent wash with sterilized distilled water. These pieces were kept onto PDA medium in Petri dishes. Plates were incubated at $27 \pm 2^{\circ}\text{C}$ for 2 day. The pathogen was identified as *S. rolfsii* based on its mycelial and sclerotial characteristics (Barnett and Hunter, 1972).

Experimental setup

The experiment was conducted in Plant Pathology laboratory of Agriculture and Forestry University (AFU), Chitwan, Nepal. Approximately 20-ml of autoclaved media was poured in each petri plate (9-cm diameter). Each plate was inoculated with a 5-mm diameter mycelial plugs from the margins of 3-days old culture grown on PDA. Plates were then incubated at $27 \pm 2^{\circ}\text{C}$ with 12-hour photoperiod for two weeks. The radial mycelial growth was measured in every 2 days interval for 14 days. The experiment was replicated four times.

Data analysis

Analysis of variance (ANOVA) was conducted using R version 3.6.0. Significant differences between treatments were determined using a Duncan multiple range test (DMRT) at $p=0.05$.

III. RESULTS AND DISCUSSION

Mycelial growth

Although the mycelial growth of *S. rolfsii* in papaya dextrose agar was significantly lower than potato dextrose agar up to 6 days of incubation, the mycelial growth was at par in both growth media after 8-DAI (Table 2). In chickpea dextrose agar medium, the mycelial growth was consistently superior over the 14-DAI (Table 2). This result showed that chickpea dextrose agar, papaya dextrose agar and bean dextrose agar can be used as alternative nutrient source for the growth of *S. rolfsii*.

Since sugars are the principle constituents of papaya with total content of 48.3% sucrose, 29.8% glucose and 2% fructose (Chan and Kwok, 1975; Gomez et al., 2006) and it also contains sodium, potassium, Magnesium, Calcium, Iron and other vitamins which encourages the growth of pathogen.

Chickpea contains on an average 23% of protein, 64% carbohydrate, 6% crude fibre and 3% ash. It also has high mineral content like phosphorous, calcium, magnesium, iron and zinc etc. these nutrients are capable of supporting fungal growth (Junakali AK et al., 2009).

Kan A et al. (2010) reported that chickpea has natural antibacterial activity against gram negative bacteria like *Staphylococcus aureus*, *Bacillus substilis* and *Enterococcus faecalis*. Hence the natural antibacterial property in Chickpea dextrose agar has additional benefits as media more selective for fungi and less prone to bacterial contamination.

Table 2: Effect of different growth media on the mycelial growth of the pathogen *S. rolfsii* Sacc.

Treatments	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI
Papaya dextrose agar	1.25 ^{cd}	1.70 ^d	2.47 ^{cd}	3.22 ^{bcd}	6.10 ^b	8.10 ^a	9.00 ^a
Chickpea dextrose agar	2.45 ^a	3.35 ^a	4.50 ^a	5.37 ^a	7.30 ^a	7.95 ^a	8.77 ^{ab}
Potato dextrose agar	2.17 ^{ab}	3.02 ^{ab}	3.50 ^b	3.97 ^b	5.52 ^b	7.35 ^{ab}	7.87 ^{abc}
Bean dextrose agar	1.75 ^{bc}	2.65 ^{bc}	3.32 ^b	3.80 ^{bcd}	5.87 ^b	6.97 ^{ab}	7.57 ^{bcd}
Sabouraud dextrose agar	1.05 ^d	1.62 ^d	2.10 ^d	2.65 ^d	4.45 ^c	6.12 ^{bcd}	6.90 ^{cd}
Carrot dextrose agar	0.92 ^d	1.07 ^e	1.30 ^e	1.55 ^e	3.85 ^c	5.05 ^{cd}	6.47 ^d
Czapek Dox Agar	1.97 ^{ab}	2.32 ^c	2.95 ^{bcd}	3.05 ^{cd}	3.82 ^c	4.20 ^d	4.55 ^e
F test	***	***	***	***	***	***	***
SEM	0.119	0.139	0.237	0.239	0.529	0.821	0.613
LSD _{0.001}	0.025	0.027	0.035	0.035	0.053	0.066	0.052
CV (%)	20.8	16.6	16.9	14.5	13.7	13.8	10.7

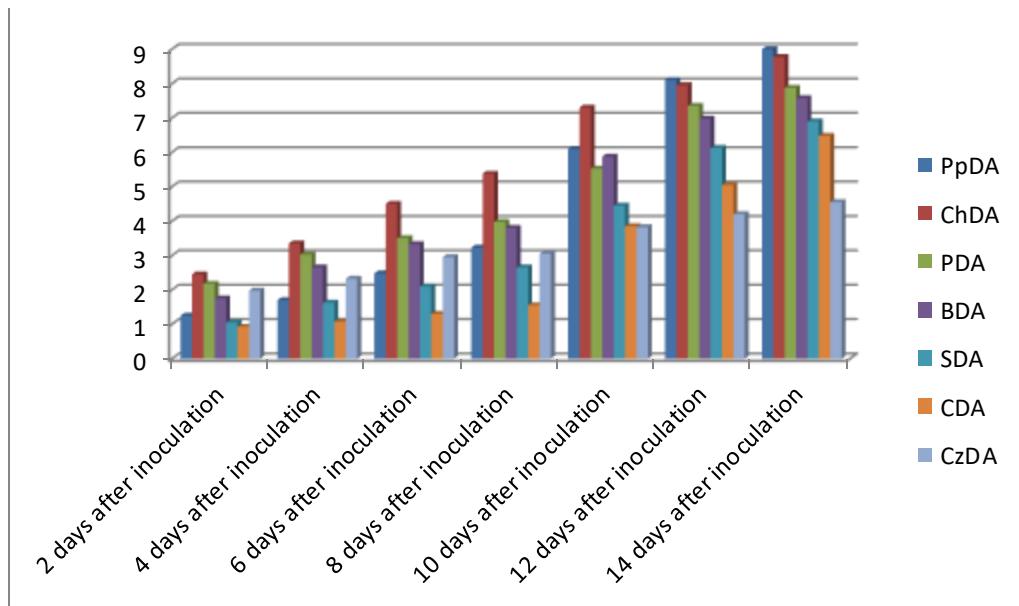


Fig.1: Mycelial growth of *S. rolfsii* in different culture media at various days after incubation

Mycelium characteristics on different media

The mycelium grown in chickpea dextrose agar (ChDA) had bright white color and Papaya Dextrose Agar (PpDA) had dull white color. Chickpea dextrose agar (ChDA), Bean Dextrose Agar (BDA) had condensed mycelium whereas uncondensed in Carrot Dextrose Agar (CDA).

Table 3: Comparison of mycelium characters of *S. rolfsii* on different growth media

SN	Media	Growth	Colony color	Appearance
1	PpDA	Initially slow and very fast in later	dull white	Cottony dull white
2	ChDA	Moderate	Bright white	Dense cottony white
3	PDA	Moderate	Cottony white	Medium Cottony white
4	BDA	Moderate	Light white	Condensed cottony
5	SDA	Slow to moderate	Bright white	Moderately condensed
6	CDA	Slow to fast	Bright white	Uncondensed cottony
7	CzDA	Slow	Extra white	Wavy like, condensed at margins, aggregated

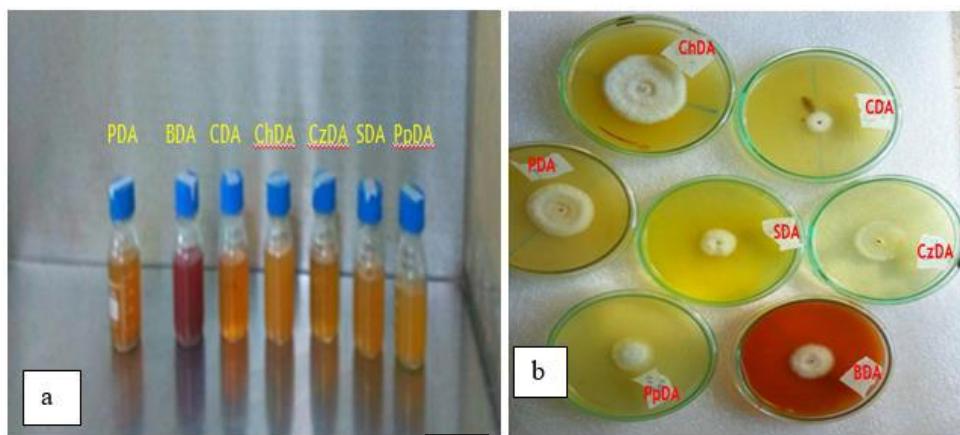


Fig.2: Preparation of culture media (a) and mycelial growth of *S. rolfsii* in different culture media at 6 DAI

IV. CONCLUSION

Potato dextrose agar (PDA) is most commonly used fungal media. As an alternative to PDA, Papaya dextrose agar (PpDA), Chickpea dextrose agar (ChDA) and Bean dextrose agar (BDA) can be used for the culture of sterile fungi like *S. rolfsii*. Papaya, chickpea and bean can be the cheap source of carbon for manufacturing the fungal culture media. Further study should be done to know the sporulation of fungi in these natural media.

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